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Immunology in Defiance: A Clinician's Perspective on Modern Organ Transplantation

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Abstract

Organ transplantation began experimentally early in the 20th century, following the pioneering microsurgical work of Alexis Carrel, and has since evolved into a modern clinical reality. Indeed, contemporary medicine with its immunomodulation armamentarium has triumphed over many challenges to usher in an area of successful organ transplantation that has prolonged the lives of millions. The current practice of transplant surgery includes the transfer of tissues, partial organs and whole organs, including the heart, liver, kidneys, pancreas and lungs. In addition to whole organs, successful bone, heart valve, cartilage, vein and artery, and cornea transplantations are performed with increasing frequency and clinical acumen. Nevertheless, inherent physiological and immunological problems exist in the field of organ transplantation, regardless of the organ or tissue involved, and these problems must be successfully overcome if a transplant, and in many cases the patient, are to survive and function optimally. Delineating the mechanisms underpinning acute and chronic rejection has become a focus of recent research groups, but determining the pathophysiological mechanisms is not enough in this era of evidence-based medicine; clarifying the implications of the immune rejection of transplanted organs for society at large does, indeed, conjure images of immunology in defiance.

Keywords: Transplantation; Immunology; Heart; Lung; Liver; Kidney; Rejection

The laws of transplantation and the physiological and immunological risks

The eminent immunologist George Schone cogently summarized the insights of late 19th century medical science in 1912 and in doing so elegantly defined the classic "laws of transplantation" that, in the absence of immune involvement, still apply today [1]. Contemporary research clearly upholds these fundamental principles, but formulates them in a more precise way, which can be expressed as the following:

Transplantation in the absence of immunomodulation into a foreign species invariably fails. Transplantation of allograft in to unrelated members of the same species usually fails. Auto grafts almost invariably succeed. There is a primary "take" and then delayed rejection of the first graft into an unrelated member of the same species. There is accelerated rejection of a second graft in a recipient that had previously rejected a graft from the same donor, or of a first graft in a recipient that had been pre-immunized with material from the same donor.

The closer the blood relationship between donor and recipient, the higher the success rate at least in clinical terms. These guiding principles still guide the clinician and provide a framework with which those in the basic sciences can formulate future experiments [1,2].

Rejection of non-self, disparate tissues is mediated by histocompatibility antigens that are products of genetic transcription; the loci that initiate the most aggressive forms of allograft rejection are inherently linked with the major histocompatibility complex (MHC). Although other antigens initiate less intense reactions, myriad combinations of antigens have been demonstrated to provoke and potentiate organ and tissue rejection. Major histocompatibility complex molecules have been divided into two clear classes, each with clear delineating features.

The seminal work of Alexis Carrel in microsurgery and the transfer of tissues based on a demonstrable vascular lifeline suggested the possibility of organ transplantation, but it was the work of others in subsequent decades that made it a clinical reality. The first successful organ transplant, performed in 1954 by Joseph Murray, involved identical twins; as such, immune rejection was not observed [3]. The surgical replacement of diseased organs with healthy ones has helped prolong the lives of millions of patients, but necessitates specialized immunosuppressive therapy after transplantation to maximize graft survival. Chief to this immunomodulation is the attenuation of natural killer (NK) cell function [3,4]. Much research has focused on the role of NK cells in tolerance induction, but it is important to appreciate the myriad contributions of these cells to both acute and chronic graft rejection [3,5]. Indeed, NK cells are thought to potentiate MHC-disparate hematopoietic stem cell rejection, thereby constituting a vital impediment to T cell–directed tolerance. It has also been postulated that NK cells are not sufficient to mediate allograft rejection independent of normal functioning immunity, but play a key role in the process by interacting with various other cell types and inflammatory cytokines in an 'immune soup'[5].

The biochemical basis of immunological defense and inflammation

Immunoglobulin's is specifically modified proteins present in serum and tissue fluids, which are capable of selectively reacting with antigens and initiating or potentiating an immune reaction. It is well accepted that antibodies largely have the same basic structure, and that each antibody is specific and can recognize only one antigen, to which it binds with great avidity [6]. The immune system generates several million antibodies that are capable of reacting with several millions antigens. Antibodies are involved in a wide range of immune responses that lead to the destruction and elimination of potentially harmful antigens. Consequently, antibodies are formed and attracted to these foreign structures for which they have identical matching receptors. In this way, antibodies bind with antigens, forming antigen-antibody complexes, which is a precise, highly regulated process [5,6].

Inflammation is the body's attempt to restore homeostasis, or its internal milieu; it is the initial reaction to injury and the first step in the healing process. Wound healing cannot occur if the inflammatory

response is fully inhibited. There are a series of cellular and systemic reactions that are triggered during the inflammatory response that localize and destroy the offending antigen, which maintain vascular integrity, and thereby limit tissue damage [7]. The inflammatory response can be altered or suppressed with the administration of corticosteroids and other immunosuppressive agents, as well as malnutrition, advanced age and concomitant illnesses, especially diabetes, autoimmune conditions and active malignancy [7,8].

Histocompatibility and immunogenicity of transplant rejection

Histocompatibility is a measure of how well two tissues coexist in the same biologic environment. The major physiologic barrier in transplantation is the potential for rejection of transplanted organs as a result of the normal process of immunity; the recognition of non-self antigens and the attenuation of the resultant immunological cascade is at the very heart of current immunomodulation with its growing array of immunosuppressant agents [8]. Tissues transplanted from one individual to another, in the absence of immunosuppression, will be rejected if the recipient's immune system recognizes the transplanted organ or tissue as foreign. Histocompatibility testing is used to minimize graft "foreignness" and reduce donor-specific immune responses to the transplanted organ [8,9]. Foreignness is equated with the presence on transplanted tissue membrane of antigens that the host does not possess and therefore recognizes as non-self. Previous studies have clearly demonstrated that if all other factors are optimal (e.g., donor management, the functional state of the donor organ, the surgical procedure, and intraoperative management of the recipient), the major reason for transplant failure is rejection [9].

There are different types of rejection, depending on the timing, but many studies suggested that the T cell-mediated immune response has a major role in rejection of transplanted organs [9].

Antigen presenting cells (APC) express MHC molecules that bind to non-self and present thermoses that T- T-cell MHC n rejection of transplanted organs (Ref) molecules and present them to T cells, initiating a T cell-mediated immune response. Upon transplantation, donor organs express MHC molecules and the resulting immune response can generally be of two types; one direct and the other indirect. The direct pathway refers to the reaction between allogenic APC/MHC/peptide complexes and recipient T cells, whereas the indirect pathway involves allogenic MHC molecules, which are themselves recognized by the recipient's APC and subsequently presented to the host's T cells. Two types of glycoprotein are expressed on the surface of the T cell; CD4 (helper T cell) and CD8 (cytotoxic T cell). The CD4 T cell mediated immune response is responsible for delayed-type hypersensitivity and is known to be one of the major contributing factors for chronic rejection; hence the many drug targets to suppress T cell activity.

It is perhaps pertinent to now further examine the role of NK cells in more detail. Natural killer cells are potent cytolytic cells that induce tissue inflammation by releasing a host of pro-inflammatory cytokines, including IL-1, IL-6 and TNF alpha [3,4]. What is clear from the literature is the key role of NK cells as 'effectors cells' in transplant rejection. Their other important subsidiary role is to up regulate inflammatory cytokines and other associated cells, including lymphocytes and monocytes, thereby potentiating the immune response [4]. Recent studies, however, have demonstrated additional roles for NK cells in the induction of transplant tolerance [3-5]. Indeed, it has been found that NK cells control survival of graft-derived donor cells by inhibiting alloreactive T cells. In other studies, NK cells are found to regulate the induction of regulatory T cells, which is important for tolerance induction in transplant patients [3,4]. It can therefore be appreciated that NK cells are intimately involved in both graft rejection and tolerance induction; these apparent antagonistic roles may be mediated by differences in the activation status of NK cells, which is ultimately driven by altered gene expression and affected by the balance of the other immune cells.

It is increasingly well recognized that a thorough pre-transplant assessment can reduce the risk of rejection. A prospective lymphocyte cross-matching program has been performed on a regular basis as described by Patel and Terasaki, where the rate of hyper acute allograft rejection has been significantly reduced [10]. In addition, many different trials were performed in order to increase the detection sensitivity of cross-matching techniques. There are other new techniques that have been developed for pre-transplant assessments, such as donor-reactive HLA-specific and IgG antibody analysis, which demonstrates a high sensitivity in determining the risk of organ rejection in renal transplant recipients [11]. Furthermore, the risk can be categorized into negligible, intermediate or high risk, and as such, post-transplant management is tailored according to patients' risk profile according to the pre-transplant assessment [11].Despite these purported advantages, transplantation, by its very nature, imposes an inherent risk of rejection. Indeed, non-detectable HLA antibodies do not confer a complete freedom from potential rejection and/or graft failure due to the following 1) antibody was not detected because of the lack of sample availability to test, 2) concentration of antibody is below detectable range, or 3) memory B cells/T cells are present without antibody present [11]. It is this last factor that is perhaps the most difficult to overcome.

Hyperacute, acute and chronic rejection: a problem of timing

Rejection is an adaptive immune response via cellular immunity, mediated by cytotoxic lymphocytes, including T cells, which induces apoptosis of target cells, as well as humoral immunity, mediated by activated immunoglobulin-secreting B cells [12]. Augmenting these two processes are the components of innate immune system, namely complement and phagocytes. Hyper acute rejection, by its very definition, causes great anxiety for practicing clinicians; transplanted tissues are rejected within minutes to hours owing to a rapidly progressive ischemia characterized by a direct, humorally-mediated vascular insult. The speed with which this process can occur signifies the importance of preexisting antibodies, which directly target the graft. Sensitizing events include previous pregnancy or blood transfusions, as well as previous xenotransplantation [12,13].

The implicated pathomechanics involves the dynamic interplay between antigen-antibody complexation and the secondary activation of the complement system, leading to capillary and arteriolar thrombosis and subsequent progressive ischemia. Moreover, preformed donor-specific antibodies that are the consequence of the adaptive immune response drive this response. Hyper acute rejection is an antibody-mediated cytotoxic response to the fixation of antibodies to specific class I antigens on vascular endothelium, followed by entrapment of formed blood elements and clotting factors in the microvasculature of the graft, resulting in complement activation, massive intravascular coagulation with a concomitant consumptive coagulopathy, as well as diminished tissue perfusion, and

ultimately, graft necrosis [12,13]. Hyper acute rejection results in immediate thrombotic occlusion and loss of the allograft, particularly in heart transplants, because of the intricate vasculature of the myocardium, but is also observed in other solid organ transplants [1,2,12]. If the involved organ is left implanted in the patient, then a systemic inflammatory response syndrome (SIRS) can develop, which often necessitates management in the intensive care unit. Moreover, if the donor organ is lost, then re-transplantation becomes necessary to prolong the life of the patient.

Indeed, from the surgeon's point of view, hyper acute rejection can occur while the patient is still in the operating room, and this of course represents a clinical disaster. All major organs are susceptible to this form of rejection, although the reason why hyper acute rejection does not readily occur in liver grafts is not fully understood; it is speculated that the enormous cell mass of the liver is capable of absorbing circulating antibodies. Another reason may be differences in micro vascular structure [12-14]. Hyper acute rejection exemplifies the humoral response, which is developed through an earlier primary exposure that primed specific immunity to the non-self antigen. It can therefore be appreciated that transplant patients can have a multitude of immunoglobulin cross-reacting with the donor tissue upon transplantation, which represents a secondary exposure event. At this secondary exposure, these cross-reactive antibodies dynamically interact with complement and phagocytes, which are soluble immune complexes and innate immune cells generated by an activated immune system, respectively; the sum of these events is the loss of a transplanted organs structure and function.

The manifestation of acute rejection is generally held to be within the first six months following transplantation [4]. There are two primary mechanisms responsible for acute rejection, chiefly acute cellular rejection, involving mononuclear and cytotoxic cells, and humoral rejection. Activated lymphocytes drive the cellular response, following lymphoid tissue sensitization, which implies a temporal limit in the nature of this response. Donor dendritic cells serve as antigen-presenting cells and have a dynamic interaction with lymphocytes. In general, a biopsy is required to make a definitive diagnosis of acute rejection, and once the diagnosis is made, emergency action can be taken, usually in the form of high-dose pulsed steroid therapy. Moreover, the concept of 'triple therapy', where a calcineurin inhibitor (cyclosporine or tacrolimus), as well as an anti-proliferative agent (azathioprine or mycophenolate) is combined with high-dose corticosteroids, has become standard practice [7]. At the cellular level, alloreactive cytotoxic T lymphocytes (CTLs) possess CD8 receptors that react with the transplanted tissue's MHC class I molecules, which display the donor's "self". Furthermore, the T cell receptors (TCRs) of the CTLs recognize their matching epitope and it is this process that triggers the target cell's apoptosis [14,15].

A single episode of acute rejection can be recognized and treated, usually preventing organ dysfunction, but multiple recurrent episodes lead to chronic rejection, which occurs months to years after transplantation, even in the presence of continued immunosuppression. The majority of kidney transplants, which now have an initial acceptance rate of 90%, inevitably fail due to the development of chronic rejection and progressive loss of function. The pathophysiological hallmark of an organ undergoing chronic rejection is fibrosis, leading to distortion of normal architecture and disordered function [4,16]. Additionally, chronic allograft vasculopathy is implicated in chronic rejection, and is especially well established in failed heart transplants; an accelerated form of atherosclerosis can be demonstrated in these hearts' coronary arteries [1,16].

Moreover, chronic rejection can explain the long-term morbidity in most lung transplant recipients, in which the median survival is generally held to be approximately half that expected of other solid organ transplants [17]. In liver transplants, chronic rejection is characterized by the "vanishing bile duct syndrome". In kidney recipients, chronic rejection (chronic allograft nephropathy) manifests as fibrosis and glomerulopathy. There are a number of factors implicated in chronic rejection, including previous episodes of acute rejection, by whatever mechanism, as well as substandard immunosuppression, prior reperfusion-ischemia injury and peri-operative nosocomial infections. Patient factors, such as hypertension and diabetes, have also been implicated in both the causation and potentiation of chronic rejection.

The main affliction of lung transplant recipients is bronchiolitis obliterans, which clinically presents as progressive airflow obstruction, leading to varying degrees of dyspnea and finally to pulmonary insufficiency, which may be associated with pneumonia and its clinical sequelae. Indeed, bronchiolitis obliterans is seen in over 50% of lung transplant recipients by five years, and in over 80% by ten years [17,18]. Histologically, there is infiltration of lymphocytes followed by injury to the epithelium and secondary inflammatory lesions, which serve to augment the pernicious affects of various pro-inflammatory cytokines, namely TNF alpha. Additionally, there is an up regulation and recruitment of fibroblasts, which potentiates this inherently fibrogenic process [18,19].

The future for organ transplantation

The transplantation of organs and tissues to cure disease has become a clinical reality. Success has been achieved as a direct result of progress in understanding the cellular and molecular biology of the immune system. This understanding has led to the development of immunosuppressive agents to combat the inherent physiological and immunological barriers. New immunosuppressive drugs are constantly under development, but organ transplantation remains a therapy that requires patients to choose between the risks of their illness and its treatment, and the risks of life-long systemic immunosuppression. Many studies have focused on minimizing the risks of rejection, while avoiding side effects of immunosuppression [20]. However, sub therapeutic levels immune suppression may cause serious side effect, such as graft rejection/dysfunction, whereas over-suppression may increase the risk of infection, cancer or toxicity [20]. Therefore, maintaining an immune tolerance with the lowest level of immunosuppressant is still the biggest challenge of post-transplant care. A number of studies have investigated the efficacy of different combinations and monotherapy, as well as the timing of reducing the dose, yet outcomes remain controversial [20].

Although rejection cannot be completely prevented, a degree of immune tolerance to the transplant does develop in most cases. There exist a number of proposals that explain this phenomenon. It is increasingly being recognized that clonal deletion and the development of immunologic energy in donor-specific T and B cells, together with the development of "suppressor lymphocytes" leads to the attenuation of the immune response. Moreover, this down-regulation of the immune response, despite the presence of non-self tissues, gives hope for both transplant patients and clinicians. Another paradigm maintains that the up regulation of donor-derived dendritic

cells in the recipient generates a finely balanced state of chimerism, in which self and non-self are immunologically indistinguishable.

Conclusion

Foreseeable alternatives to immunosuppression include modulation of donor grafts to reduce immunogenicity and the induction of a state of immunologic tolerance, but much work remains to be done to clarify whether or not lymphocytes are potential targets of this novel approach. The diagnosis of rejection relies on clinical signs and symptoms, but tissue biopsy remains the gold standard in determining whether or not an organ is undergoing rejection. Indeed, the old surgeon's adage of 'No meat, no treat' holds true in this context. The infiltration of T lymphocytes, eosinophils, plasma cells and neutrophils is associated with structural changes in the donor organ, but the concomitant vasculopathy, fibrogenesis and loss of function is what chiefly defines rejection; as immunology in defiance.

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